

**WHAT IS CLAIMED IS**

1. A multiwell plate comprising a plurality of wells wherein at the bottom surface of at least one well of said plurality of wells is a plurality of picowells.
2. The plate of claim 1, having a footprint of a standard multiwell plate.
3. The plate of claim 1, wherein said plurality of wells comprises  $6n$  wells arranged in a  $2n \times 3n$  array, where  $n$  is an integer greater than 0.
4. The plate of claim 1, wherein wells of said plurality of wells are rectangularly packed.
5. The plate of claim 3, wherein said plurality of wells is selected from the group consisting of 6, 24, 96, 384 and 1536 wells.
6. The plate of claim 3, wherein said plurality of wells is 96 wells.
7. The plate of claim 3, wherein said plurality of wells is 384 wells.
8. The plate of claim 1, wherein said plurality of picowells comprises individually addressable picowells.
9. The plate of claim 1, wherein the bottoms of all picowells in a said well of the plate are substantially coplanar.
10. The plate of claim 1, wherein the bottoms of all picowells of the plate are substantially coplanar.
11. The plate of claim 1, wherein picowells of said plurality of picowells are juxtaposed.

- 54 -

12. The plate of claim 11, wherein the interwell area between two said picowells is less than about 0.35 the sum of the areas of said two picowells.

13. The plate of claim 11, wherein the interwell area between two said picowells is less than about 0.25 the sum of the areas of said two picowells.

14. The plate of claim 11, wherein the interwell area between two said picowells is less than about 0.15 the sum of the areas of said two picowells.

15. The plate of claim 11, wherein the interwell area between two said picowells is less than about 0.10 the sum of the areas of said two picowells.

16. The plate of claim 11, wherein the interwell area between two said picowells is less than about 0.06 the sum of the areas of said two picowells.

17. The plate of claim 11, wherein a rim of a said picowell is substantially knife-edged.

18. The plate of claim 1, wherein said plurality of picowells comprises picowells having dimensions of less than about 200 microns.

19. The plate of claim 18, wherein the dimensions of said picowells are less than about 100 microns.

20. The plate of claim 18, wherein the dimensions of said picowells are less than about 50 microns.

21. The plate of claim 18, wherein the dimensions of said picowells are less than about 25 microns.

22. The plate of claim 18, wherein the dimensions of said picowells are less than about 10 microns.

- 55 -

23. The plate of claim 1, wherein said plurality of picowells comprises picowells configured to hold no more than one living cell of a certain size.

24. The plate of claim 1, wherein said plurality of picowells comprises picowells configured to hold no more than a predetermined number of living cells of a certain size.

25. The plate of claim 1, wherein picowells of said plurality of picowells comprise enclosures of dimensions such that substantially an entire cell of a certain size is containable within a said enclosure, each said enclosure having an opening, said opening defined by a first cross section of a size allowing passage of a cell of a certain size.

26. The plate of claim 25, wherein the volume of a said enclosure is less than about  $1 \times 10^{-11}$  liter.

27. The plate of claim 25, wherein the volume of a said enclosure is less than about  $1 \times 10^{-12}$  liter.

28. The plate of claim 25, wherein the volume of a said enclosure is less than about  $1 \times 10^{-13}$  liter.

29. The plate of claim 25, wherein the volume of a said enclosure is less than about  $1 \times 10^{-14}$  liter.

30. The plate of claim 25, wherein the volume of a said enclosure is less than about  $1 \times 10^{-15}$  liter.

31. The plate of claim 25, wherein the area of a said first cross section is less than about 40000 micron<sup>2</sup>.

- 56 -

32. The plate of claim 25, wherein the area of a said first cross section is less than about 10000 micron<sup>2</sup>.

33. The plate of claim 25, wherein the area of a said first cross section is less than about 2500 micron<sup>2</sup>.

34. The plate of claim 25, wherein the area of a said first cross section is less than about 625 micron<sup>2</sup>.

35. The plate of claim 25, wherein the area of a said first cross section is less than about 100 micron<sup>2</sup>.

36. The plate of claim 25, a said enclosure having dimensions so as to contain no more than one cell of said certain size at any one time.

37. The plate of claim 25, a said enclosure having dimensions so as to contain no more than a predetermined number of cells of said certain size at any one time.

38. The plate of claim 1, said plurality of picowells comprising picowells, wherein all picowells of the plate are substantially identical in size.

39. The plate of claim 1, wherein a first said well includes a first said plurality of picowells and a second said well includes a second said plurality of picowells, wherein said first plurality of picowells and said second plurality of picowells are substantially different.

40. The plate of claim 39, wherein the size of picowells of said first plurality of picowells and the size of picowells of said second plurality of picowells are substantially different.

- 57 -

41. The plate of claim 1, comprising a material selected from the group consisting of ceramics, elastomers, epoxies, glasses, glass-ceramics, metals, plastics, polycarbonates, polydimethylsiloxane, polyethylenterephthalate glycol, polymers, polymethyl methacrylate, polystyrene, polyurethane, polyvinyl chloride, rubber, silicon, silicon oxide and silicon rubber.

42. The plate of claim 1, said bottom surface comprising a material selected from the group consisting of ceramics, elastomers, epoxies, glasses, glass-ceramics, metals, plastics, polycarbonates, polydimethylsiloxane, polyethylenterephthalate glycol, polymers, polymethyl methacrylate, polystyrene, polyurethane, polyvinyl chloride, rubber, silicon, silicon oxide and silicon rubber.

43. The plate of claim 1, wherein the walls of wells of said plurality of wells are integrally formed with said bottom surface.

44. The plate of claim 1, further comprising at least one distinct well-wall component attached to said bottom surface.

45. The plate of claim 44, said distinct well-wall component comprising a material selected from the group consisting of ceramics, elastomers, epoxies, glasses, glass-ceramics, metals, plastics, polycarbonates, polydimethylsiloxane, polyethylenterephthalate glycol, polymers, polymethyl methacrylate, polystyrene, polyurethane, polyvinyl chloride, rubber, silicon, silicon oxide and silicon rubber.

46. The plate of claim 1, wherein said plurality of picowells are integrally formed with said bottom surface.

47. The plate of claim 1, further comprising at least one distinct picowell-bearing component bearing said plurality of picowells attached to said bottom surface of said one well.

- 58 -

48. The method of claim 47, wherein said picowell-bearing component is a carrier comprising a plurality of picowells disposed on a surface.

49. The plate of claim 47, said distinct picowell-bearing component made of a material selected from the group consisting of reversibly deformable materials and irreversibly deformable materials.

50. The plate of claim 47, wherein said distinct picowell-bearing component is made of a material selected from the group consisting of waxes, hydrocarbon waxes, crystalline waxes, paraffins, ceramics, elastomers, epoxies, glasses, glass-ceramics, metals, plastics, polycarbonates, polydimethylsiloxane, polyethylenterephthalate glycol, polymers, polymethyl methacrylate, polystyrene, polyurethane, polyvinyl chloride, rubber, silicon, silicon oxide and silicon rubber.

51. The plate of claim 1, further comprising at least one distinct picowell-bearing component bearing said plurality of picowells resting within said one well.

52. The method of claim 51, wherein said picowell-bearing component is a carrier comprising a plurality of picowells disposed on a surface.

53. The plate of claim 51, wherein said distinct picowell-bearing component is made of a material selected from the group consisting of reversibly deformable materials and irreversibly deformable materials.

54. The plate of claim 51, wherein said distinct picowell-bearing component is made of a material selected from the group consisting of gels, hydrogels, waxes, hydrocarbon waxes, crystalline waxes, paraffins, ceramics, elastomers, epoxies, glasses, glass-ceramics, metals, plastics, polycarbonates, polydimethylsiloxane, polyethylenterephthalate glycol, polymers, polymethyl methacrylate, polystyrene, polyurethane, polyvinyl chloride, rubber, silicon, silicon oxide and silicon rubber.

- 59 -

55. The plate of claim 51, wherein said picowell-bearing component comprises a gel.

56. The device of claim 55, wherein said gel is substantially transparent.

57. The device of claim 55, wherein said gel is a hydrogel.

58. The device of claim 55, wherein the water content of said gel is greater than about 80% by weight.

59. The device of claim 55, wherein the water content of said gel is greater than about 92% by weight.

60. The device of claim 55, wherein the water content of said gel is greater than about 95% by weight.

61. The device of claim 55, wherein the water content of said gel is greater than about 97% by weight.

62. The device of claim 55, wherein the water content of said gel is greater than about 98% by weight.

63. The device of claim 55, wherein said gel is made of a material selected from the group consisting of agar gels, agarose gels, gelatins, low melting temperature agarose gels, alginate gels, room-temperature  $\text{Ca}^{2+}$ -induced alginate gels and polysaccharide gels.

64. The device of claim 55, wherein said gel comprises an active entity.

65. The device of claim 64, wherein said active entity is selected from the group consisting of antibodies, antigens, biological materials, chemical materials, chromatogenic compounds, drugs, enzymes, fluorescent probes, immunogenes,

- 60 -

indicators, ligands, nucleic acids, nutrients, peptides, physiological media, proteins, receptors, selective toxins and toxins.

66. The plate of claim 1, said plurality of picowells comprising picowells, said picowells having a bottom surface made of a first material and borders made of a second material, said second material being substantially different from said first material.

67. The plate of claim 66, wherein said first material is substantially the material from which said bottom of said well is made.

68. The plate of claim 66, wherein said bottom surface of said picowell is substantially said bottom surface of said well.

69. The plate of claim 66, wherein said second material is fixed photoresist material.

70. The plate of claim 1, wherein said plurality of picowells comprises picowells having an inside surface configured to delay adhesion of living cells thereto.

71. The plate of claim 70, wherein said inside surface comprises an adhesion-delaying material.

72. The plate of claim 70, wherein said inside surface is coated with said adhesion-delaying material.

73. The plate of claim 1, said plurality of picowells comprising picowells, the bottom of said picowells substantially having an index of refraction similar to that of water.

74. The plate of claim 73, wherein said index of refraction is less than about 1.4.

75. The plate of claim 73, wherein said index of refraction is less than about 1.38.
76. The plate of claim 73, wherein said index of refraction is less than about 1.36.
77. The plate of claim 73, wherein said index of refraction is less than about 1.35.
78. The plate of claim 73, wherein said index of refraction is less than about 1.34.
79. The plate of claim 1, said plurality of picowells comprising picowells, said picowells having an inner surface coated with a layer of a material.
80. The plate of claim 79, said material selected from the group consisting of gels, hydrogels, polydimethylsiloxane, elastomers, polymerized para-xylylene molecules, polymerized derivatives of para-xylylene molecules, rubber and silicon rubber.
81. The plate of claim 1, further comprising a gel cover covering said plurality of picowells.
82. The plate of claim 1, wherein said plurality of picowells covers substantially the entire said bottom surface of said well.
83. The plate of claim 1, further comprising at least one additional feature functionally associated with said plurality of picowells.
84. The plate of claim 83, wherein said additional feature comprises a microfluidic feature functionally associated with said plurality of picowells.

- 62 -

85. The plate of claim 84, wherein said feature is selected from the group consisting of channels, coupling elements, drains, fluid channels, fluid reservoirs, input ports, membranes, microreactors, microvalves, output ports, passages, plumbing routes, protruberances, pumps, transport channels and valves.

86. The plate of claim 83, wherein said feature is selected from the group consisting of light sources, magnetizable elements, optical components, optical fibers, optical filters, protuberances, fiducial points and walls.

87. The plate of claim 1, further comprising a cover slip, said cover slip and said plurality of picowells configured so as to allow said cover slip to rest above said plurality of picowells substantially in parallel to said bottom surface.

88. A method of making a multiwell plate of claim 1, comprising:

(a) contacting a precursor material with a template including a negative of features of the plate so as to create said features in said precursor material, said features including said plurality of picowells;

(b) fixing said features in said precursor material so as to fashion an incipient plate; and

(c) processing said incipient plate so as to fashion the plate.

89. The method of claim 88, wherein said template comprises a material selected from the groups consisting of reversibly deformable materials, irreversibly deformable materials, ceramics, epoxies, glasses, glass-ceramics, metals, plastics, polycarbonates, polydimethylsiloxane, polyethylenterephthalate glycol, polymers, polymethyl methacrylate, paraffins, polystyrene, polyurethanes, polyvinyl chloride, silicon, silicon oxide, silicon rubbers and wax.

90. The method of claim 88, wherein said fixing comprises a method selected from the group consisting of heating said precursor material, cooling said precursor material, polymerizing said precursor material, cross-linking said precursor material, curing said precursor material, irradiating said precursor material, illuminating

- 63 -

said precursor material, gelling said precursor material, exposing said precursor material to a fixative and waiting a period of time.

91. The method of claim 88, said features further comprising at least one feature selected from the group of features consisting of channels, coupling elements, drains, fluid channels, fluid reservoirs, input ports, microreactors, microvalves, passages, optical components, optical filters, output ports, plumbing routes, protruberances, pumps, transport channels, valves, walls, patrial walls and fiducial points.

92. The method of claim 88, said features further comprising said plurality of wells.

93. The method of claim 88, further comprising:

(d) prior to (a), placing said precursor material in a well of a multiwell plate.

94. The method of claim 88, further comprising:

(d) subsequent to (b), attaching walls of said plurality of wells to said incipient plate.

95. The method of claim 94, wherein said attaching comprises using an adhesive to attach said walls to said incipient plate.

96. The method of claim 94, wherein said attaching comprises using a surface treatment to attach said walls to said incipient plate.

97. The method of claim 96, wherein said surface treatment is a plasma treatment.

98. The method of claim 88, wherein said precursor material includes a irreversibly deformable precursor material and said fixing said features comprises separating said template from said precursor material.

- 64 -

99. The method of claim 98, wherein said irreversibly deformable precursor material is selected from the group consisting of wax, paraffins, plastics and polymers.

100. The method of claim 88, wherein said precursor material comprises an reversibly deformable precursor material.

101. The method of claim 100, wherein said reversibly deformable precursor material is selected from the group consisting of gellable fluids, polymerizable materials, powders, fluids and thermoplastic materials.

102. The method of claim 101, wherein said reversibly deformable precursor material includes a thermoplastic material at plastic temperature and wherein said fixing said features comprises cooling said thermoplastic material.

103. The method of claim 101, wherein said reversibly deformable precursor material includes a polymerizable material and wherein said fixing said features comprises polymerizing said polymerizable material.

104. The method of claim 103, wherein said polymerizable material is selected from the group consisting of monomer solutions, crosslinkable polymers, vulcanizable polymers, polymerizable fluids and thermosetting resins.

105. The method of claim 104, wherein said polymerizable material includes a polydimethylsiloxane precursor mixture and said fixing said features comprises polymerizing said polydimethylsiloxane precursor mixture so as to produce polydimethylsiloxane.

106. The method of claim 104, wherein said polymerizable material includes urethane and said fixing said features comprises polymerizing said urethane to produce polyurethane.

- 65 -

107. The method of claim 101, wherein said reversibly deformable precursor material includes a gellable fluid and wherein said fixing said features comprises gelling said gellable fluid.

108. The method of claim 107, wherein said gelling said gellable fluid comprises an action selected from the group consisting of heating said gellable fluid, cooling said gellable fluid, irradiating said gellable fluid, illuminating said gellable fluid, contacting said gellable fluid with a gelling reagent and waiting a period of time for said gellable fluid to gel.

109. The method of claim 107, wherein said gellable fluid comprises a solution including a material selected from the group consisting of agars, agaroses, gelatins, low melting temperature agaroses, alginates, proteins, protein polysaccharides,  $\text{Ca}^{2+}$ -inducable alginates and polysaccharides.

110. The method of claim 107, wherein said gellable fluid includes an alginate solution and said gelling said gellable fluid comprises contacting said gellable fluid with a gelling reagent.

111. The method of claim 110, wherein said gelling reagent comprises  $\text{Ca}^{2+}$  ions.

112. The method of claim 107, wherein said gellable fluid includes a low melting temperature agarose solution and said gelling said gellable fluid comprises cooling said gellable fluid.

113. The method of claim 88, wherein said processing said incipient plate comprises coating an inside surface of picowells of said plurality of picowells with a layer of a coating material.

114. The method of claim 113, wherein said coating said inside surface comprises:

- 66 -

(i) applying a precursor fluid with said inside surface of said picowells; and

(ii) solidifying said precursor fluid so as to form said layer of said coating material.

115. The method of claim 114, wherein said solidifying comprises a method selected from the group consisting of heating said precursor fluid, cooling said precursor fluid, polymerizing said precursor fluid, cross-linking said precursor fluid, curing said precursor fluid, irradiating said precursor fluid, illuminating said precursor fluid, gelling said precursor fluid, exposing said precursor fluid to a fixative and waiting a period of time.

116. The method of claim 113, wherein said coating said inside surface comprises:

(i) depositing a vapor of said coating material onto said inside surface of said picowells thereby forming said layer of said coating material.

117. The method of claim 113, wherein said coating said inside surface comprises:

(i) depositing a vapor of a coating precursor material onto said inside surface of said picowells; and

(ii) solidifying said coating precursor material thereby forming said layer of said coating material.

118. The method of claim 117, wherein said solidifying said coating precursor material comprises a method selected from heating said coating precursor material, cooling said coating precursor material, polymerizing said coating precursor material, cross-linking said coating precursor material, curing said coating precursor material, irradiating said coating precursor material, illuminating said coating precursor material, gelling said coating precursor material, exposing said coating precursor material to a fixative and waiting a period of time.

- 67 -

119. The method of claim 117, wherein said vapor is a vapor of para-xylylene molecules or derivatives thereof and said layer of said coating material comprises a layer of polymerized said para-xylylene molecules or derivatives thereof.

120. A method of making a multiwell plate of claim 1, comprising:

- (a) placing a photoresist material on a precursor plate; and
- (b) fixing a plurality of picowells in said photoresist material.

121. The method of claim 120 wherein said fixing comprises irradiating said photoresist material through a mask.

122. The method of claim 120, wherein said precursor plate comprises a material selected from the group consisting of ceramics, epoxies, glasses, glass-ceramics, metals, plastics, polycarbonates, polydimethylsiloxane, polymers, polyethylenterephthalate glycol, polymethyl methacrylate, polystyrene, polyurethanes, polyvinyl chloride, silicon and silicon oxide.

123. The method of claim 120, wherein said precursor plate comprises a multiwell plate.

124. The method of claim 120, further comprising:

- (c) subsequent to (b), attaching walls of wells to said precursor plate.

125. The method of claim 120, further comprising:

(c) subsequent to (b), coating an inside surface of picowells of said plurality of picowells with a layer of a coating material.

126. A method of making a multiwell plate of claim 1, comprising placing a picowell-bearing component on a precursor plate.

- 68 -

127. The method of claim 126, further comprising attaching said picowell-bearing component to said precursor plate

128. The method of claim 127, wherein said attaching comprises using an adhesive to attach said picowell-bearing component to said precursor plate.

129. The method of claim 127, wherein said attaching comprises a surface treatment to attach said picowell-bearing component to said precursor plate.

130. The method of claim 129, wherein said surface treatment is a plasma treatment.

131. The method of claim 126, wherein said precursor plate comprises a multiwell plate.

132. The method of claim 126, further comprising attaching walls of wells to said precursor plate.

133. The method of claim 126, wherein said picowell-bearing component is a carrier comprising a plurality of picowells disposed on a surface.

134. The method of claim 126, further comprising, subsequent to said attaching of said picowell-bearing component, coating an inside surface of picowells of said picowell-bearing component with a layer of a coating material.

135. A device comprising an array of living cells held in a non-fluid matrix, said matrix configured to maintain cell viability.

136. The device of claim 135, wherein said living cells are physically held in pockets in said matrix.

- 69 -

137. The device of claim 135, whercin there is substantially no bond between said living cells and said matrix.

138. The device of claim 135, wherein said array is substantially planar having an upper surface and a lower surface.

139. The device of claim 138, wherein at least one said surfaces is transparent to at least one wavelength of light.

140. The device of claim 139, wherein a said transparent surface is said upper surface.

141. The device of claim 139, wherein a said transparent surface is said lower surface.

142. The device of claim 139, wherein said at least one wavelength of light is in the ultraviolet spectrum.

143. The device of claim 139, wherein said at least one wavelength of light is in the visible spectrum.

144. The device of claim 139, wherein said at least one wavelength of light is in the infrared spectrum.

145. The device of claim 135, said matrix comprising a material having an index of refraction substantially similar to that of water.

146. The device of claim 145, said matrix comprising a material having an index of refraction less than about 1.4.

147. The device of claim 145, said matrix comprising a material having an index of refraction less than about 1.38.

- 70 -

148. The device of claim 145, said matrix comprising a material having an index of refraction less than about 1.36.

149. The device of claim 145, said matrix comprising a material having an index of refraction less than about 1.35.

150. The device of claim 145, said matrix comprising a material having an index of refraction less than about 1.34.

151. The device of claim 135, said matrix configured to substantially delay proliferation of living cells held therein.

152. The device of claim 135, said matrix made of a material comprising a gel.

153. The device of claim 135, said matrix comprising an active entity.

154. The device of claim 153, said active entity comprising an indicator.

155. The method of claim 153, said active entity comprising an indicator, said indicator configured to indicate a cell response to a stimulus.

156. The method of claim 155, wherein said cell response is a release of a second active entity.

157. A method for handling living cells, comprising:

- (a) providing an ordered array of living cells immobilized in a non-fluid matrix, said matrix configured to maintain cell viability;
- (b) contacting said living cells with a stimulus; and
- (c) detecting a response of said cells to said stimulus.

158. The method of claim 157, said matrix comprising an active entity

159. The method of claim 158, said active entity comprising an indicator.
160. The method of claim 159, said active entity comprising an indicator, said indicator configured to indicate a cell response to a stimulus.
161. The method of claim 160, wherein said cell response is a release of a second active entity.
162. The method of claim 157, wherein said detecting comprises contacting an active entity with said matrix.
163. The method of claim 162, further comprising waiting a period of time so as to allow said active entity to reach proximity with said cells.
164. The method of claim 162, said active entity comprising an indicator.
165. The method of claim 162, said active entity comprising an indicator, said indicator configured to indicate a cell response to a stimulus.
166. The method of claim 165, wherein said cell response is a release of a second active entity.
167. The method of claim 157, wherein said detecting comprises detecting emitted light.
168. The method of claim 157, wherein said detecting comprises detecting light.
169. A method of producing an ordered array of living cells in a non-fluid matrix, comprising:

- 72 -

(a) providing a multiwell plate provided with a plurality of wells, said multiwell plate including a plurality of picowells at the bottom of at least one said well, said plurality of picowells including picowells;

(b) placing a suspension of a plurality of living cells in a gellable fluid in said at least one well;

(c) causing said living cells to settle into said picowells so as to be held in respective picowells; and

(d) gelling said gellable fluid so as to make a gel cover, trapping said living cells between said picowells and said gel cover.

170. The method of claim 169, wherein said picowells are made of a material comprising a gel.

171. The method of claim 169, wherein said causing said cells to settle comprises applying a force to said cells.

172. The method of claim 171, wherein said force is a force resulting from the impact of photons.

173. The method of claim 171, wherein said force is a force resulting from a pressure wave.

174. The method of claim 171, wherein said force is gravitation.

175. The method of claim 171, wherein said force is a centrifugal force.

176. The method of claim 169, wherein

(e) prior to (d), ensuring that substantially each picowell holds no more than one living cell.

177. The method of claim 169, wherein said gelling said gellable fluid comprises an action selected from the group consisting of heating said gellable fluid,

- 73 -

cooling said gellable fluid, irradiating said gellable fluid, illuminating said gellable fluid, contacting said gellable fluid with a gelling reagent and waiting a period of time for said gellable fluid to gel.

178. The method of claim 169, wherein said gellable fluid is selected so as to form a hydrogel upon said gelling.

179. The method of claim 169, wherein said gellable fluid is selected from solutions containing a material selected from the group consisting of agars, agaroses, gelatins, low melting temperature agaroses, alginates, room-temperature  $\text{Ca}^{2+}$ -inducable alginates and polysaccharides.

180. The method of claim 179, wherein said gellable fluid is an alginate and said gelling said gellable fluid comprises contacting said gellable fluid with a gelling reagent.

181. The method of claim 179, wherein said gellable fluid is a low melting temperature agarose and said gelling said gellable fluid comprises cooling said gellable fluid.

182. The method of claim 169, wherein said gellable fluid comprises an active entity.

183. The method of claim 169, said active entity comprising an indicator.

184. The method of claim 183, said active entity comprising an indicator, said indicator configured to indicate a cell response to a stimulus.

185. The method of claim 184, wherein said cell response is a release of a second active entity.